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Attorney Docket No.: 5694.200-US PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Flodgaard et al.

Serial No.: 09/559,764 Group Art Unit: 1644

Filed: April 27, 2000 Examiner: Jessica H. Roark

Confirmation No: 2707

For: Inhibition Of Bradykinin Release

DECLARATION UNDER 37 C.F.R. 1.132

Commissioner for Patents
Washington, DC 20231

Sir:

I, Thomas Renné do declare that:

1. I am a collaborator but not an inventor of one of the inventors, Dr. Hans J. Flodgaard. My Curriculum Vitae is attached.

2. I have reviewed the application and Office Action issued in connection with the above-referenced application. In particular, I have reviewed the section of the Office Action on page 4 which states:

3. The experiments described below address this issue and were done under my direction and in collaboration with Dr. Hans Flodgaard.

4. To analyze the binding of HBP to kininogen, high molecular weight kininogen (HK) competition assays were performed. Heparan sulfate (HS) was covalently immobilized on microtiter wells (A, C, D) and competition assays with biotinylated HK (biot-HK) (10 nM) as probe were performed. Biot-HK bound to these 'artificial cell surfaces' was detected with a streptavidin point of detection (POD) detection system and quantified photometrically.

Received from < 9147120094 > at 8/29/02 5:19:27 PM [Eastern Daylight Time]

5. The following experimental procedure was used for the HK competition assays. For all *in vitro* competition analyses, 250 µg/ml HS was covalently immobilized to CovaLink™ microtiter plates (Nunc, Wiesbaden, Germany) using 0.05 M N-hydroxysuccinimide (NHS, Pierce, St. Augustin, Germany) and 0.2 M 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC, Pierce) for the coupling procedure as described (Renne, T., et al., J. Biol. Chem. 275:33688). Biot-HK (10 nM) was incubated together with a serial 1:2 dilution, of the GAG binding proteins HBP, heparin rich glycoprotein (HRG), FXII or casein (starting concentration 1µM). Incubation was done in HEPES-Tyrode's buffer, (HT) with 1 % (m/v) bovine serum albumin (BSA) supplemented with 10 µM $ZnCl_2$, respectively. Specifically bound biot-HK was detected by the preformed biotin-streptavidin-peroxidase complex (2 µg/ml; Boehringer Mannheim, Germany) followed by chromogene substrate 0.15% (w/v) diammonium 2,2'-azido-bis-(3-ethyl-2,3-dihydrobenzthiazoline-6-sulfonate) (ABTS).

6. The Results are shown in the attached Figure. The insert at the top of *A* depicts the setup of the assay: the filled triangle symbolizes the competitor, the filled triangle at the top of the stem, the covalently immobilized HS and the open polygon represents Biot-HK. Similar results were obtained when the assay was performed on confluent EA.hy926 cells (B). The experimental setup is identical as (A); the open ellipse symbolizes the endothelial cells. (*A, B*) GAG binding proteins HBP (□), HRG (○), FXII (△) applied in a serial 1:2 dilution series (starting from 20 µM) competed with biot-HK for binding to HS whereas casein (■) failed to detach HK.

7. These results do indicate that HBP displaces HK assembled on the surface of immobilized heparan sulfate or endothelial cels better than histidine-rich glycoprotein, factor XII or HK itself.

8. I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and

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further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 28.9.'02

DR. THOMAS RENNÉ

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LEBENSLAUF

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AUSBILDUNG

07/1975-07/1979	Grundschule Staudernheim.
08/1979-06/1988	Staatliches Gymnasium Sobernheim, Abitur
07/1988-09/1989	Wehrdienst beim JaBoG 35 Pferdsfeld.
10/1989	Beginn des Medizinstudiums an der Johannes Gutenberg-Universität Mainz.
10/1990-07/1994	Chemiestudium an der Johannes Gutenberg-Universität Mainz (Studiendauer 8 Semester).
08/1994-08/1995	Diplomarbeit "Lokalisation einer H-Kininogen Bindungsstelle in der schweren Kette von Kallikrein" am Institut für Physiologische Chemie und Pathobiochemie, Universität Mainz (Prof. Dr. W. Müller-Esterl).
08/1995	Diplom Chemie
09/1995-02/1999	Promotionsarbeit am Institut für Physiologische Chemie und Pathobiochemie (Prof. Dr. W. Müller-Esterl) mit dem Thema "Molekulare Mechanismen Kinin-generierender Systeme".
08/1996	1. Staatsexamen Humanmedizin (Note: befriedigend).
07/1999	Promotion Chemie
03/1999-12/2001	"PostDoc" am Institut für Biochemie II, Klinikum der Johann Wolfgang Goethe-Universität Frankfurt (Prof. Dr. W. Müller-Esterl).

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	Projekt: "Freisetzung von Bradykinin bei Entzündungen und im Rahmen der Hämostase".
08/2000	2. Staatsexamen Humanmedizin.
10/2000-10/2001	Praktischen Jahres am Klinikum der Johannes Gutenberg-Universität Mainz.
11/2001	3. Staatsexamen Humanmedizin.
01/2002	Arzt im Praktikum am Institut für Klinische Biochemie und Pathobiochemie der Bayerischen Julius-Maximilians-Universität Würzburg (Prof. Dr. U. Walter)

AUSLANDSAUFENTHALTE

08/1992-03/1993	Kyoto University, Kyoto, Japan, Department of Bioorganic Chemisty (Prof. Dr. H. Ogoshi).
02/2001-06/2001	Vanderbilt University, Nashville, TN, USA, Hematology Division (Prof. Dr. D. Gailani).

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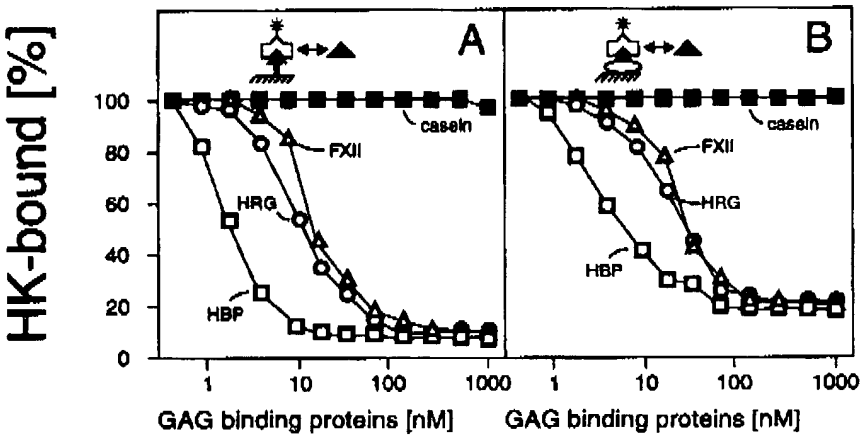

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